

AMENDMENTS

Listing of Claims

The following listing of claims replaces all previous listings or versions thereof:

1. (Original) A method of assaying for binding of a test compound to a phosphodiesterase (PDE) comprising:
 - (a) providing a PDE;
 - (b) mixing said PDE with a positively-charged peptide or polypeptide and a test compound;
 - (c) passing the mixture of step (b) through a filter;
 - (d) washing said filter with an ionic detergent solution; and
 - (e) measuring test compound associated with said filter,

wherein test compound retained on said filter indicates binding of said test compound to said PDE, and wherein said binding of said test compound to PDE is measured at or near stoichiometric levels.

2. (Original) The method of claim 1, wherein said PDE is PDE1, PDE2, PDE3, PDE5, PDE6, PDE7, PDE8, PDE9, PDE10 or PDE11.
3. (Original) The method of claim 1, wherein said PDE is PDE2, PDE5, PDE6 or PDE11.
4. (Original) The method of claim 1, wherein said test compound is labeled, and measuring comprises assessing filter-associated label.
5. (Currently amended) The method of claim 4, further comprising performing on said test compound a similar control reaction having steps (b)-(e) of claim 1 wherein said mixture lacks a-PDE is absent from step (b).

6. (Currently amended) The method of claim 4, further comprising performing on a similar control reaction ~~wherein said test compound is substituted~~having steps (a)-(e) of claim 1 with a known PDE-binding compound.
7. (Original) The method of claim 6, wherein said known PDE-binding compound is sildenafil, vardenafil or cGMP, and said PDE is PDE5.
8. (Original) The method of claim 4, wherein said label is a radioactive label, a fluorescent label, a dye, a chemilluminous label, an enzymatic label, or a ligand.
9. (Original) The method of claim 8, wherein said label is a radioactive label.
10. (Original) The method of claim 9, wherein said radioactive label is ^3H .
11. (Original) The method of claim 8, wherein said label is a fluorescent label.
12. (Original) The method of claim 11, wherein said fluorescent label is fluorescein, rhodamine, green fluorescent protein, and red fluorescent protein.
13. (Original) The method of claim 8, wherein said label is a chemilluminous label.
14. (Original) The method of claim 13, wherein said chemilluminous label is luciferase.
15. (Original) The method of claim 1, wherein step (b) is performed at less than 15°C.
16. (Original) The method of claim 15, wherein step (b) is performed at about 4°C.
17. (Original) The method of claim 1, further comprising pre-wetting said filter with an ionic detergent solution.
18. (Original) The method of claim 1, wherein said ionic detergent solutions comprises Triton X-100.
19. (Original) The method of claim 8, wherein measuring comprises scintillation counting.
20. (Original) The method of claim 1, wherein said PDE is mixed with said positively-charged peptide or polypeptide prior to mixing with said test compound.

21. (Original) The method of claim 1, wherein said PDE is derived from a tissue extract.
22. (Original) The method of claim 1, wherein said PDE is recombinant PDE.
23. (Original) The method of claim 1, wherein said PDE is purified PDE.
24. (Original) The method of claim 1, wherein said positively-charged peptide or polypeptide is a histone.
25. (Original) The method of claim 1, wherein the mixture of step (b) further comprises or is further mixed with a known PDE-binding compound that is labeled, and measuring comprises assessing filter-associated label, wherein a reduction in filter-associated label, as compared to a similar control reaction lacking said test compound, indicates binding of said test compound to said PDE.
26. (Original) The method of claim 25, wherein said known PDE-binding compound is sildenafil, vardenafil or cGMP, and said PDE is PDE5.
27. (Original) The method of claim 25, wherein said label is a radioactive label, a fluorescent label, a dye, a chemilluminous label, an enzymatic label, or a ligand.
28. (Original) The method of claim 27, wherein said label is a radioactive label.
29. (Original) The method of claim 28, wherein said radioactive label is ³H.
30. (Original) The method of claim 27, wherein said label is a fluorescent label.
31. (Original) The method of claim 30, wherein said fluorescent label is fluorescein, rhodamine, green fluorescent protein, and red fluorescent protein.
32. (Original) The method of claim 27, wherein said label is a chemilluminous label.
33. (Original) The method of claim 32, wherein said chemilluminous label is luciferase.
34. (Original) The method of claim 25, further comprising performing a similar control reaction wherein said mixture lacks said test compound.

35. (Currently amended) The method of claim 25, further comprising performing on said test compound and said known PDE-binding compound a similar control reaction having steps (b)-(e) of claim 1 wherein said mixture lacks a PDE is absent from step (b)
36. (Original) The method of claim 1, wherein said filter is a paper filter, a nitrocellulose filter, a glass microfiber filter or a quartz microfiber filter.
37. (Original) The method of claim 36, wherein said paper filter is a Whatman 0.45 μ m filter.